Embryonic and yolk-sac larval development of saddled bream, *Oblada melanura* (Sparidae)

by

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ABSTRACT. - The embryonic and yolk-sac larval development of laboratory-reared saddled bream *Oblada melanura* (Linnaeus, 1758) is described. The fertilized eggs were pelagic and transparent with one oil globule with a mean diameter of 0.833 ± 0.055 mm and a range from 0.755 to 0.928 mm. Embryonic development lasted 32 h 30 min at 21.9 ± 0.4ºC. Newly hatched yolk-sac larvae were 1.992 ± 0.111 mm in length. The yolk-sac was completely absorbed after 56 hours, when larvae reached 3.050 ± 0.041 mm. The mouth started to open 48 h after hatching and was functional after 60 h with an opening diameter of 0.180-0.210 mm. Changes in length and shape of yolk-sac larvae during the first three days after hatching are also presented.

RÉSUMÉ. - Développement des embryons et des larves vitellines de l'oblade *Oblada melanura* (Sparidae).

Le développement embryonnaire et celui des larves vitellines de l'oblade, *Oblada melanura* (Linnaeus, 1758), élevée en laboratoire, sont décrits. Les œufs fertilisés sont pélagiques et transparents, contiennent une gouttelette d'huile, et possèdent un diamètre de 0,755 à 0,928 mm, avec une moyenne de 0,833 ± 0,055 mm. Le développement embryonnaire a duré 32 h 30 à une température de 21.9 ± 0.4ºC. Les larves nouvellement écloses mesuraient 1,992 ± 0,111 mm de longueur totale. Le vitellus a été complètement résorbé 56 heures après l'éclosion, quand les larves atteignaient en moyenne 3,050 ± 0,041 mm. La bouche larvaire a commencé à s'ouvrir 48 h après l'éclosion, et elle était fonctionnelle après 60 h. La bouche avait alors un diamètre de 0,180-0,210 mm. Les changements de longueur et de forme des larves vitellines pendant les trois premiers jours après l'éclosion sont également présentés.

Key words. - Sparidae - *Oblada melanura* - Embryo - Yolk-sac larvae - Development.

The saddled bream, *Oblada melanura* (Linnaeus, 1758), is common throughout the Mediterranean and Atlantic (from the Bay of Biscay to Angola, Madeira, the Canaries and Cape Verde Islands), inhabiting littoral waters above rocky bottoms and *Posidonia oceanica* beds up to depths of 30 m (Bauchot and Hureau, 1986). It is also common in the Adriatic Sea (Jardas, 1996). The scientific literature pertaining to the biology and ecology of this species is scarce. Lo Bianco (1909) and Bini (1968) analyzed the growth and reproduction of saddled bream in the Adriatic Sea. Pallaoro *et al*. (1998) analyzed biological parameters of the saddled bream in the eastern Adriatic.

Saddled bream is the object of intensive fishing in the eastern Adriatic and represent about 78.6% of the catches of gear "ludar" (fishing with nets using ropes), which is common in Croatian fishing areas (Cetinić and Pallaoro, 1993; Pallaoro and Cetinić, 1993).

Data on eggs size of mature embryo and yolk-sac larval development of *O. melanura* in available literature is limited, and is generally related to the Tyrrhenian Sea and the western Mediterranean. Raffaele (1888) describes the size and shape of embryos. De Gaetani (1931) describes yolk-sac larvae at the moment of hatching. Holt (1899; cited in Lo Bianco, 1909) discussed egg morphology, and Lo Bianco (1909) described the larval life history after metamorphosis. Significantly more research has been done on other species of sparids. A great deal of research has been done on the embryonic and yolk-sac larval development of sparids, such as *Diplodus vulgaris* (Jug-Dujaković and Glamuzina, 1988), *Dentex dentex* (Jug-Dujaković *et al*., 1995), *Pagrus pagrus* (Radonić *et al*., 2005; Kentouri *et al*., 1992; Mihelakakis *et al*., 2001; Mylonas *et al*., 2004), *Pagrus major* (Hattori *et al*., 2004), *Lithognathus mormyrus* (Firat *et al*., 2005),

The embryonic and larval stages constitute a critical period during fish life history, in which understanding the biology and ecology is important to adjust culture conditions to the status of embryo and larvae. For this reason, both the success and progress of larviculture depend specially on an adequate knowledge of the development of embryo and larvae under controlled conditions.

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The identification of early stages is critical for the systematic study of egg and larval abundance in population estimates.

This paper describes the first data on embryonic and yolk-sac larval development in controlled laboratory conditions of *Oblada melanura*. The objective was to describe the early life history and assist in the identification of the planktonic stages, thus contributing to ichthyoplankton studies.

MATERIAL AND METHODS

Broodstock (30 females and 14 males) was collected from eastern Adriatic waters and was held in experimental hatchery tanks at the Institute of Marine and Coastal Research in Dubrovnik at temperature of 21.8 ± 0.9ºC, salinity 37.2 ± 0.6 psu and natural photoperiods, 10 h dark-14 h light. Sex ratio was 2.1:1 in favors of females. Body weight of individual fish ranged from 190.0-375.0 g and fish were 5-8 years old. From all recaptured fish scale were sampled from a standard area (Ombredane and Baglinière, 1992). Scale from each fish were read only once using a microfiche reader (50x), and age was designated by a standard notation following Baglinière and Le Louarn (1987) and Richard and Baglinière (1990). Fish were hand-fed twice a day to satiation with European pilchard (*Sardina pilchardus*). Two months before the start of the spawning season, they were given a moist pellet diet supplemented with vitamins and minerals. Gonad tissue samples were taken once monthly from all broodstock specimens, starting in March, in order to monitor all gonad changes prior and during the spawning season. Ovarian biopsy was obtained using tygon cannula (1.88 mm o.d. x 1.11 mm i.d.), as described by Shehedeh *et al*. (1973). Females were selected for induced ovulation based on their oocyte development. Candidates for induced ovulation were initially chosen based on maximum oocyte diameters measured to the nearest 0.025 mm using a stereo microscope fitted with measurement devices (Wild Heerbrugg type 325400). Four vitellogenic females with maximum oocyte diameters of 0.450-0.550 mm were anaesthetised in a bowl using a benzocaine solution 1 mg/L. Anaesthetised females were given injections of LHRH-a 20 μg/kg, and a second injection of 20 μg/kg after 24 h. After 48 h, females were stripped in a clean and dry bowl. Milt was taken from several males using sterilized syringes, taking care to maintain a dry anal area. Milt was then spread over the eggs and carefully mixed. Sea water was added to activate the spermatozoa. Temperature of the sea water was ambient. After 15 min, fertilized eggs were separated from those unfertilized, based on their floatation (fertilized eggs float). Before they were separated to several 60L incubators, eggs were disinfected with 400 ppm glutaradehyde for 10 min (Salvesen and Vadstein, 1995). Temperature of treated sea water (filtration and ultraviolet (UV) sterilization) in incubator was 21.9 ± 0.4ºC, salinity 37.2 ± 0,7 psu and photoperiod was natural, 10 h dark-14 h light. Each aerated incubator received 100 mL of 2 μm filtered air per minute through an air stone (3 x 1.5 cm) placed on the bottom of the incubator (Komar *et al*., 2004). The number of eggs/mL was estimated based on counts of 1 mL sub-samples (n = 5) of floating eggs. Embryonic development was observed under a stereo microscope and pictures of individual stages were taken. The egg diameter was measured 30 min after fertilization. A sample of (n = 30) eggs was taken every 5-7 min to determine the exact time of first cleavage. Embryogenesis was examined at different time intervals. Yolk-sac larvae (n = 30) were anaesthetized in a bowl using benzocaine 0.05 mg/mL. Anaesthetized yolk-sac larvae in live conditions were measured to an accuracy of 0.01 mm using an ocular micrometer attached to a stereo microscope.

Table I. - Embryonic development of saddled bream, *Oblada melanura*, at a mean temperature of 21.9 ± 0.4ºC.

Time	Stage	Description
0:00	Fertilization	
0:55	2 cells	Meridional first cleavage
1:26	4 cells	Second cleavage
1:49	8 cells	
2:08	16 cells	Cleavage parallel to the second
2:24	32 cells	Cleavage parallel to the first
2:48	64 cells	Morula start
3:30	Blastula	
4:31	Early gastrula	Gastrulation starts
4:56	Middle gastrula	
9:26	Late gastrula	
16:00	Neurula	Formation of embryo begins, notochord develops
16:49	Embryo	Formation of optic vesicle
17:19		Appearance of Kuppfer's vesicle
17:37		Formation of seven somites
17:56		Formation of the rudimentary heart
20:58		Appearance of melanophores, elongation of tail
22:26		Appearance of melanophores on yolk sac
29:27		Heartbeat rate 72 per minute, rhythmical movements every 20 s
32:30	Free yolk-sac larvae	Hatching begins

The following measurements were taken: total length, the distance along the snout to the end of the caudal fin; notochord length, the distance along the midline from the tip of the snout to the end of the notochord; head length, the distance between the tip of the upper jaw and the cleithrum; pre-anal length, distance along the midline of the body from the tip of the snout to the vent; body depth, the perpendicular depth of the trunk at the anus; greatest body depth, body depth at its widest point; yolk-sac volume; horizontal eye diameter; mouth-width was measured, on the ventral surface, as the width between the posterior edges of the maxillae; as well as cardiac contraction. Later on, the samples were fixed with 8% buffered formalin for more detailed morphological studies. Times for yolk-sac absorption and for mouth-openings were recorded.

RESULTS

The gonad biopsy results of specimens from the broodstock showed oocyte sizes from 0.400 to 0.550 mm and good quality milt ready to use. Following treatment with LHRH-a, the eggs were ripe and with an oocyte diameter of 0.833 ± 0.055mm, ready for fertilization.

The fertilized eggs were transparent and spherical. The average fertilized egg diameter of saddled bream was 0.833 ± 0.055 mm, with a range from 0.755-0.928 mm. Developing eggs had one oil globule with an average diameter of 0.17 ± 0.003 mm. However, within one hour after fertilization, the egg diameter became more uniformed. After fertilization, several cleavages were observed in the animal pole of the egg that determined the different developmental stages. Embryonic development until hatching at 21.9 ± 0.4ºC was divided into four main stages: cleavage stage, blastula stage, gastrula stage and embryo stage. Each stage can be divided into sub-stages depending on the number of divisions that occurred or the appearance of different organs.

Table I illustrates changes observed during embryonic development at a temperature of 21.9 ± 0.4ºC. The results are organized as a staging guide. The present method for naming the stages used anatomical features.

a) Cleavage

First cleavage begins at approximately 55 min and the next four cleavage cycles occur at smaller intervals (31 min, 23 min, 19 min and 16 min interval between cleavage cycles from II to V cleavage).

b) Morula

After 32-cell stage (2:24 h) it was difficult to observe the other symmetrical division, but the cells continued to divide. During incubation, morula stage was observed at 2:48 h after the fertilization of the egg. The blastodisc consisted of many blastomeres.

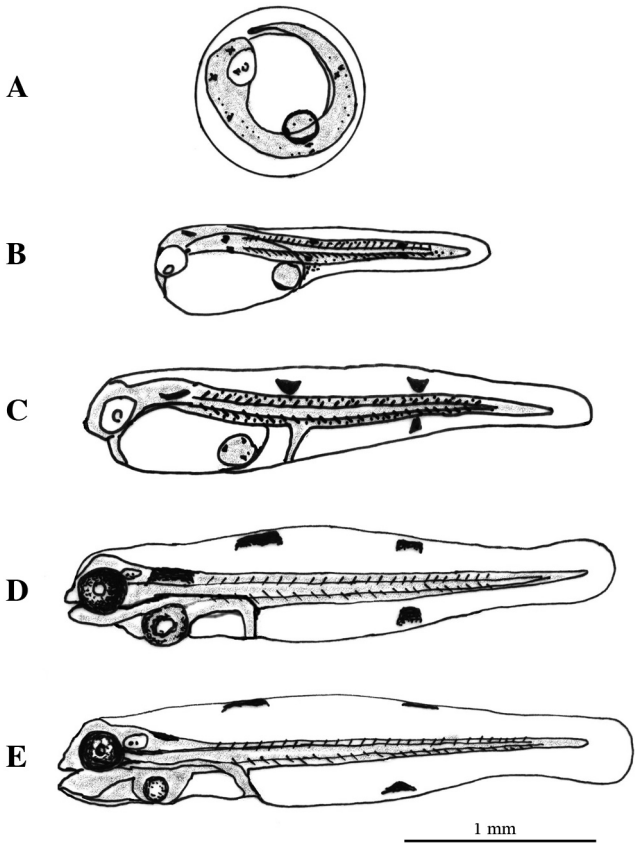

Figure 1. - **A**: Embryo 21 h after fertilization; **B**: Hatched yolk-sac larvae; **C**: Yolk-sac larvae 24 h after hatching; **D**: Yolk-sac larvae 48 h after hatching; **E**: Larvae 60 h after hatching.

c) Blastula

The blastula describes the period when the blastodisc becomes multilayered. Blastula stage lasted 1:01 h; during this period cell division becomes less synchronous. A blastocoel was formed inside the blastodisc 3:30 h after the fertilization.

d) Gastrula

During gastrula stage 4:31 h after the fertilization, the blastoderm showed true expansion as the sheet of cells extended and spread toward the vegetal pole. The blastoderm covered one-quarter of the egg and a thickened part of the blastoderm became an embryonic shield.

e) Neurula

An embryonic body was formed from the embryonic shield 16:00 h after the fertilization. Seven somites were discernible. 16:49 h after the fertilization, the brain vesicles and eye rudiment could be identified. 17:19 h after the fertilization appearance of Kuppfer's vesicle. The appearance of rudimentary heart was determined; additional 8-10 somites were observed at 17:56 h after fertilization. The appearance of first pigmentation was determined at 20:58 h after fertilization. The melanophores appeared below the oil globule

Table II. - Changes in length and shape of saddled bream, *Oblada melanura,* during the first 60 hours at a mean temperature of 21.9 ± 0.4ºC.

Hours after hatching	Total length (mm)	Notochord length (mm)	Preanal length (mm)	Head length (mm)	Eye diameter (mm)	Yolk-sac volume (mm^3)	Oil globule diameter (mm)
0	1.99 ± 0.11	1.89 ± 0.12	0.98 ± 0.11	0.25 ± 0.03	0.19 ± 0.01	0.135	0.17 ± 0.003
12	2.39 ± 0.19	2.23 ± 0.23	1.01 ± 0.01	0.28 ± 0.01	0.20 ± 0.01	0.107	0.17 ± 0.003
24	3.03 ± 0.07	2.82 ± 0.06	1.03 ± 0.13	0.33 ± 0.01	0.20 ± 0.001	0.074	0.16 ± 0.016
48	3.05 ± 0.04	2.92 ± 0.02	1.16 ± 0.05	0.36 ± 0.004	0.24 ± 0.007	0.044	0.13 ± 0.002
60	3.10 ± 0.05	2.94 ± 0.05	1.27 ± 0.03	0.43 ± 0.002	0.24 ± 0.002	absorbed	0.12 ± 0.002

Table III. - A comparison of egg sizes for various sparid species.

Species	Egg diameter (mm)	Total length of yolk-sac larvae (mm)	Reference
Sparus aurata	0.950 ± 0.018	2.58	Katavić,1984
Diplodus puntazzo	0.807 ± 0.8	1.908 ± 0.003	Kamaci *et al*., 2005
Diplodus sargus	0.975 ± 0.031	2.63	Kentouri *et al*.,1980
Diplodus vulgaris	1.010 ± 0.020	2.63	Jug-Dujaković *et.al*.,1988
Dentex dentex	0.958 ± 0.007	2.17 ± 0.2	Jug-Dujaković *et.al*.,1995
Pagrus pagrus	0.991 ± 0.1	3.178 ± 0.085	Mihelakakis *et al*., 2001
Oblada melanura	0.833 ± 0.055	1.992 ± 0.111	Present study

and along the embryonic body: no pigment spots present over the yolk, optic vesicles were developed and the tail is separated from the yolk (Fig. 1A). 22:26 h after the fertilization the heart formed; blood circulation commenced, embryos showed slight movements; the number of somites increased to 20 and melanophores appeared along the embryonic body. 29:27 h after the fertilization the heartbeat rate was 72 per min and rhythmical movements of embryos was noted.

Hatching started 32 h 30 min after fertilization. Newly hatched yolk-sac larvae measured 1.992 ± 0.111 mm in length, and ranged 1.820-2.111 mm (Fig. 1B). At the moment of hatching, the yolk-sac larva contained 28 somites: 8 abdominal and 20 caudal. The position of the oil globule was at the dorsal side of the yolk-sac. The anus was situated close behind the posterior margin of the yolk-sac. The yolk-sac larva has a double series of dark melanophores, one for each segment. It passes along the length of the dorsal body profile from the head to the end of two-thirds of the body. No melanophores were recognized over the yolk.

After 24 h from hatching (Fig. 1C), the yolk-soc larva lengthened by 50% from 1.99 mm to 3.03 ± 0.07 mm and flattened laterally, the melanophores for future dorsal and sub-tail fins thickened and increased. The eyes began to darken and became pigmented. A membranous fin was visible on the posterior body. The mouth was underdevelopment, but a straight, simple tubular gut was observed. The terminal section of the gut was about a distance equal to the diameter of the oil globule from the posterior end of the yolk-sac. The newly hatched larvae remained suspended in the water and quivered occasionally.

After 48 h from hatching (Fig. 1D), the average TL was 3.05 ± 0.04 mm, and there were four remaining melanophores: three on the dorsal side; two at the position of future dorsal fins, the third at the start of the posterior part of the head, and one at the ventral side at the position of future sub-tailfins. The mouth was under development and is invaginated but not yet opened. Although the yolk sac was not completely absorbed, the different parts of the alimentary canal (esophagus, stomach and intestine) began to differentiate. Faint granular pigmentation of the eye was completed.

After 60 h from hatching (Fig. 1E), the average TL was 3.10± 0.05 mm and the yolk-sac larva grew by one third from its initial length (Tab. II), the eye pigmentation is complete, the yolk-sac absorbed and the oil globule is reduced to 0.12 mm and is closer to the heart. Mouth is open and functional; mouth width was 180-210 μm.

DISCUSSION

The spawning season of broodstock of saddled bream was the same as in wild populations. In the wild, the onset of spawning season of saddled bream is from the end of May to the start of August (Jardas, 1996; Pallaoro, 1996). All males in captivity had mature milt during the entire spawning season, while captive females showed progress in oocyte sizes before hormone treatment. Synthetic analogues of LHRHa which we used have been effectively used to induce ovulation and spawning in a number of commercially important finfish including Sparidae species such as gilthead seabream, *Sparus aurata* (Zohar, 1986). Hormonally treated females spawned eggs similar in size, shape and structure to those of other sparids. Common saddled bream egg is a typical pelagic sparid egg with one oil globule. One oil globule for other sparid eggs was reported by Jug-Dujaković *et al*. (1995); Radonić *et al*. (2005); Faranda *et al*. (1985); Kentouri *et al*. (1980); Kamaci *et al*. (2005). Our results fit the data given by

De Gaetani (1931) for saddled bream in Naples Bay, where pelagic-sized eggs were 0.88 to 1.000 mm in size, with one oil globule 0.020 mm in diameter. The average egg size of saddled bream in our research is smaller than for some other species of sparids such as: *Sparus aurata* (Katavić, 1984), *Diplodus puntazzo* (Kamaci *et al*., 2005), *Diplodus sargus* (Kentouri *et al*., 1980), *Diplodus vulgaris* (Jug-Dujaković and Glamuzina, 1988), *Dentex dentex* (Jug-Dujaković *et al*., 1995), *Pagrus pagrus* (Mihelakakis *et al*., 2001) (Tab. III). According to Kamaci *et al*. (2005), egg diameter of sparids eggs can be used for species identification in theory; however, this is not very reliable in practice, as egg size can differ among females of the same species and may be dependent upon female age, spawning time and geographical origin. The level of relative variation of egg size in marine fish populations is shown to be consistent across a wide taxonomic range of species and much of this initial variation appears to be due to maternal effects (Chambers and Leggett, 1996).

Embryonic development of saddled bream is greatly influenced by temperature (Lasker, 1981). Comparison to common sea bream *Pagrus pagrus*, where is hatching time 30 h at 20ºC (Radonić *et al*., 2005) which is significantly shorter to our time (32:30 h) if you take into consideration that incubation temperatures of saddled bream were higher by almost 2ºC.

Cleavage stage is characterized by early cleavages that occur synchronously; time between I and V cleavage is reduced. First cleavage occurred at the animal pole 55 min after spawning. The egg showed a typical discoidal cleavage and two blastomeres of equal size were formed. During blastula stage there was not evidence of any abnormal blastomeres. Blastomere morphology is a useful tool for routine fish egg checking in hatcheries because of the fact that abnormal blastomeres yield low egg viability (Radonić *et al*., 2005).

The length of newly hatched yolk-sac larvae of saddled bream was significantly less (t-test, $p < 0.05$) than those of the other four species *Sparus aurata, Diplodus sargus, Diplodus vulgaris, Pagrus pagrus* (Tab. III). This information may assist in identifying newly hatched yolk-sac larvae; however, it would be of limited value since during the first few hours after hatching yolk-sac larvae develop rapidly and length changes quickly (Kamaci *et al*., 2005).

Morphologically, there are few differences between the larvae of various sparid species, and similarly for their eggs. The comparison of embryonic and yolk-sac larval morphologies is very difficult when trying to identify other sparid species. The pigmentation patterns from this study and from Naples (De Gaetani, 1931) are in agreement in general. De Gaetani (1931), in his description of saddled bream yolk-sac larvae, cites two rows of melanophores, one pair for each segment, which extend from the head towards the torso up the two-thirds of the body size. Pigmentation and distribution of melanophores, which Ranzi (1930) suggested as the starting point in the diversification of early stages of sparid species, were found not to be reliable (Jug-Dujaković *et al*., 1995). The basic melanophores pattern during yolk-sac larvae development was identical in all saddled bream yolk-sac larvae. The position of three melanophores (two on dorsal and one on ventral side) (fig 1c) was consistent and could be useful for identification.

The distance of the terminal section of the gut from the posterior end of the yolk-sac was not a reliable parameter for distinguishing saddled bream yolk-sac larvae from other described sparid yolk-sac larvae. In saddled bream yolk-sac larvae 36 h after hatching, this distance is 1.5 of the oil globule diameter. In other described sparids this distance is 1.3-1.6 of the oil globule diameter after resorption of more than half of the yolk-sac content (48-72 h depending on temperature) (Jug-Dujaković *et al*., 1995).

Comparisons of embryonic and larval morphology could, in certain cases, elucidate phylogenetic relationships, and therefore contribute to taxonomical clarification (Cohen, 1985). Such comparisons should be based on larval characters that exhibit minimal flexibility to environmental cues (Dulčić *et al*., 2008). Fish identification and taxonomy are largely based on adult characteristics, and since these develop during the larval period, new characters must be discovered and validated in order to identify larval fish (Cohen, 1985).

The controlled rearing of broodstock saddled bream showed that it is possible to obtain sexually mature specimens, and hormone treatments give positive induced spawning results. A description of embryonic and yolk-sac larval development will help to better understand the biological features of early developmental stages and to better identify the species.

Acknowledgements. - We would like to thank the ministry of Science, Education and Sport of the Republic of Croatia for their financial support.

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Reçu le 14 décembre 2009.
Accepté pour publication le 3 décembre 2010.